

Amaranth Starch Isolation, Oxidation, Heat-Moisture Treatment and Application in Edible Film Formation

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Abstract— Starch was isolated from amaranth grains and subjected to modification treatments. Oxidation of isolated starch was done using sodium hypochlorite and heat-moisture treatment was done at 85°C for 6hr keeping the moisture content 30% during treatment. Native and modified starches of amaranth were used for preparation of edible films and different characteristics of films were evaluated. Both the modification treatments increased tensile strength of amaranth starch films. Heat moisture treatment increased water vapour permeability while oxidation had contrary effects on amaranth starch films. Water solubility of films of amaranth starches was reduced by modification treatments of starches. Heat moisture treatments increased yellowness of starch films.

Keywords— amaranth, edible films, heat-moisture treatment, oxidation, starch.

I. INTRODUCTION

Environmental awareness has renewed the interest in edible and biodegradable films in last few decades. Researchers are focused on developing edible films and coating based on biopolymers such as protein, starch, lipids and polysaccharides due to their environment-friendly nature, good keeping quality and safety records for food products. Among these natural polymers, starch is the important biopolymer used as thickener, emulsifier and stabilisers. Major aspects producing starch as a potential material for edible film formation are its edibility, large availability, cost effective isolation, nutritional importance, biodegradability, biocompatibility and diverse functional properties (Dang and Yoksan, 2015; Reis et al., 2015). It was concluded from different studies that edible films prepared from starch are odourless, neutral in taste, colourless, toxic free, and semi-permeable to moisture, carbon dioxide, oxygen, and lipid as well as flavour components (Shah et al., 2016). However, native starch from each source is unique in nature and have some shortcomings such as hydrophilic nature, poor mechanical strength, paste inconsistency during cooking or processing, and low freeze-thaw stability (Xie et al., 2013; Dang and Yoksan, 2015;

Sabetzadeh et al., 2015). Starches are modified by different treatments to overcome the shortcomings of native starch and to enhance the suitability for specific application. Various modification techniques such as physical, chemical, enzymatic, and genetic, or a combination of treatments have been developed to alter the properties of starches. Alteration of properties including functional, mechanical and organoleptic characteristics of starch films is possible by addition of certain amounts of various chemicals in Filmogenic solution (Mali et al., 2004), however, it is not desirable to mix chemicals to starch film for the reason that they are edible. Therefore, to improve the quality of starch films, modification of starch itself is preferable. developed oxidised and heat-moisture treated potato starch Studies conducted on development of edible films using native and modified starches of different sources revealed significant changes in various properties of films (Zavareze et al., 2012; Fonseca et al., 2015; Biduski et al., 2017). Amaranth (*Amaranthus spp.*) is a dicotyledonous plant comes under the category of pseudocereals and grown in Himalayan area and few states of India. It is well-known for good nutritional quality of its leaves and grains. Amaranth can serve as excellent source of starch due to high starch content in grains. Extremely small size of granules with diameter ranging from 1.05 to 1.78µm amaranth starch has gained attention for applications (Sindhu and Khatkar, 2016). No previous studies have been reported on the production of edible film prepared from amaranth starches modified physically and/or chemically. Therefore, present investigation was aimed on development of edible films from amaranth starch and evaluation of effects of modification treatments including heat moisture treatment and oxidation of starch on film properties.

II. MATERIALS AND METHODS

2.1 Materials

Grains of amaranth (*Amaranthus hypocondriacus*) used in this study were procured from National Bureau of Plant Genetic Resources Regional Station, Shimla, India. The

grains were screened to remove foreign matter and stored in sealed container at room temperature. The flour was prepared by grinding seeds on laboratory mill and stored in polyethylene bags at 10° C.

2.2 Starch Isolation

Starch was isolated from amaranth grains according to the alkaline steeping method (Choi et al., 2000). Grains were steeped in 0.25% aqueous NaOH solution for 18 hr at room temperature and stirred three times during this period. After steeping, the grains were washed with distilled water and ground in a blender at full speed for 2 min, and slurry was filtered step wise through 100 mash (150µm), 270 mesh (53µm) and 400 mesh (38 µm) sieves. The starch was isolated from the filtrate by centrifugation at 25,000g for 20 min. The supernatant was discarded, and the top yellowish layer of protein was removed. This step was repeated to obtain a white starch layer. The starch layer was re-suspended in distilled water, shaken and centrifuged as described above. Thereafter, the isolated starch was dried in hot air oven at below 40°C for 8 to 10 hr and stored at room temperature in sealed container.

2.3 Heat Moisture Treatment of Starch

The heat moisture treatment of amaranth starch was carried out according to the method of Franco et al. (1995) with minor modifications. The moisture level of starch was adjusted to 30% by adding appropriate volume of distilled water (the moisture level of native starch was predetermined). The addition of distilled water was done slowly and simultaneously mixed for uniform distribution of water in starch powder. Sample was sealed in polyethylene pouch and equilibrated at 10°C overnight. After the incubation, starch was filled in air tight glass container and heated for 6 hr at 85°C. The container was shaken occasionally for uniform distribution of heat. The sample was cooled to room temperature and dried at 40° C for 6 to 8 hr and equilibrated at room temperature for 4hr. The dried starch powder was sealed in polyethylene bag, labelled and stored at room temperature for further analysis.

2.4 Oxidation of Starch

Oxidation of isolated starch was done by following the method of Forssell et al. (1995). Starch sample was weighed 100g (db) and dispersed in 500ml distilled water. The pH of the suspension was adjusted to 9.5 with 2.0M NaOH. Sodium hypochlorite solution (4% active chlorine available) of volume 25ml was slowly added to the starch slurry over a period of 30min with constant stirring while maintaining the pH in the range from 9.0 to 9.5 with 1M H₂SO₄. The reaction was allowed for 10min after all the sodium hypochlorite has been added. The pH of slurry was adjusted to 7 with 1M H₂SO₄ and centrifuged at 4000rpm for 10 min. The starch cake obtained was

washed 4 to 5 times with distilled water and dried at 40°C in hot air oven. The dried oxidized starch was ground and passed through 75µm sieve, packed in polyethylene bags, labelled and stored at room temperature for further analysis.

2.4.1 Carboxyl content of starch

The carboxyl content of the oxidized starch was determined by following the method described by Smith (1967) and employed by Parovuori et al. (1995). In 500 mg starch (db) sample, 30 ml of 0.1 M HCl was mixed at room temperature to acidifying the carboxyl groups of the samples and maintained under the magnetic stirring for 30 min. Subsequent to this, the starch was exhaustively washed and recovered by centrifugation (2,000g) until the pH raised to neutrality. This procedure was named demineralization by Smith (1967). The starch was dispersed in 300 ml of distilled water and heated at 98°C for 30 min under agitation for complete starch gelatinization. While still hot, the samples were titrated with 0.002 M NaOH solution until pH 8.3, using the phenolphthalein as indicator. Complete experiment was performed with native starch instead of oxidised starch and treated as blank. The carboxyl content was calculated using the following equation:

$$\text{COOH (\%)} = \frac{(V_s - V_b) \times M \times 0.045 \times 100}{\text{wt. of sample (g)}}$$

Where V_s is the volume of NaOH used for the sample (ml); V_b is the volume of NaOH used for the blank (ml); M is the molarity of NaOH.

2.5 Preparation of Starch Films

Starch films using native and modified starches of amaranth were prepared by following the method described by Chandla et al. (2017) with minor modifications. Filmogenic solutions were prepared by dispersion of 5g starch in 100 ml distilled water with continuous stirring at magnetic stirrer for 15min. Glycerol at rate of 3g/100g starch was added as plasticizer and mixed thoroughly. The solution was magnetically stirred for 15min at 85°C. The resulting solution was cooled at room temperature to avoid air bubbles during pouring. Casting technique was used to prepare films. The prepared solution was poured onto the polypropylene round trays of diameter 12.5cm and dried at 40°C for 16hr in hot air oven with circulating air in chamber.

2.6 Analysis of Films

2.6.1 Thickness

The thickness of starch films was determined using Digital micrometer with an accuracy of ±0.001mm. The average value of 10 thickness measurement at different locations on each film was used in all calculations.

2.6.2 Moisture Content

Moisture content of starch films was determined by drying the pre-weighed pieces of films at 110°C for 6 to

8hr or till the weight comes constant. Moisture content was measured as loss in weight of film sample during heating.

2.6.3 Water Solubility

Water solubility of starch films was determined by following the method of Gontard et al. (1994). Pre-weight piece of starch film was immersed in water at room temperature for 24hr. The immersed film piece was removed from water and dried in oven at 110°C for 4 to 5 hr, cooled and weighed. The water solubility of starch film was measured as the difference in weight of dried piece of film before and after immersion in water.

2.6.4 Color Parameters

Color of native and modified starch films was measured using CR-300 Chroma meter (Minolta, Japan). The system determines the L*, a* and b* values, where L* represents lightness and darkness; a* represents the opposition between green and red color ranging from positive (red) to negative (green) values; and b* is the yellow/blue opposition ranging from positive (yellow) to negative (blue) values. The average value of three measurements were calculated and used.

2.6.5 Water vapour permeability

Water vapour permeability of starch films was determined by following the E96-95 ASTM standard method (ASTM, 1995). Each film sample was sealed over the circular opening of a permeation cell containing anhydrous CaCl₂ (0% RH) and weighed. These cells were placed on desiccators with a saturated NaCl solution (75% RH) at 25°C. The weight of each permeation cell was recorded after 24hr and water vapour permeability of films was calculated using following formula-

$$WVP = \frac{\Delta W \times X}{t \times A \times \Delta P}$$

Where WAP is the water vapour permeability (g.mm/m².day.kPa); ΔW is the weight gain by desiccant (g); X is the film thickness (mm); t is the incubation period (days); A is the area of the exposed film surface (m²); and ΔP is the difference of partial pressure (kPa).

2.6.6 Tensile Strength

Tensile strength of films was determined by a tensile test based on ASTM D-882-91 method (ASTM, 1996) using texture analyser (TA-XT 2i Stable Micro Systems, UK). The films were cut in strips (20mm × 50mm) and thickness of strips was measured at eight points. The strip was gripped from both the edges of width on 'tensile grip' probe and initial grip separation was set at 30mm. The force and distance were recorded during extension of strips at 0.8mm/s up to break. The tensile strength of films was calculated using following formula-

$$TS = \frac{F}{A}$$

Where TS is the tensile strength (MPa); F is the maximum force (N); A is the area of film cross-section (thickness × width; m²).

2.7 Statistical Analysis

Analytical determinations were done in triplicate, and Duncan test was conducted to examine significant difference among experimental mean values. The statistical significance was observed at p < 0.05. Data were analyzed using Statistical Analysis System SAS, version 8.2 and SPSS software version 16.0 (SPSS Inc).

III. RESULTS AND DISCUSSION

Oxidized starch showed 0.099% carboxyl content which was higher than that of 0.052% reported by Fonseca et al. (2015) in potato starch. Oxidation of starch depends on various factors such as reaction conditions (time, pH, and temperature), concentration of active chlorine and starch properties.

3.1 Thickness, Tensile Strength, Solubility and Water Vapour Permeability of Films

The film produced from the native and modified starches of amaranth were continuous and easily peelable. All film samples were visually transparent. The results of moisture content, thickness, water solubility, water vapour permeability and tensile strength of films are presented in Table 1. The moisture content of films ranged from 13.21 to 15.60% with the maximum value of oxidized starch film samples. comparatively higher range of moisture content (16 to 20.50%) was recorded by Chandla et al. (2017) for starch films of amaranth of different cultivars. Various factors including the drying temperature, relative humidity of drying chamber, starch characteristics and thickness of films affect the moisture of starch films. Higher values of moisture content of modified amaranth starch films were noticed as compared to native amaranth starch films. Increment in moisture content of heat-moisture treated starch films might be due to the increased hydrophilicity of treated starch (lower retrogradation in waxy starch) and more thickness of modified starch films than native starch film. The thickness of film is an important parameter as it influences the film properties like transparency and water vapour permeation rate. Consistency in film thickness is prerequisite for good and consistent mechanical strength attainment. Thickness of films made from native and modified starches of amaranth varied from 0.157 to 0.161mm. These thickness values were comparable with the findings of Zavareze et al. (2012) reported that the thickness of films prepared from oxidized and hydrothermally modified potato starches ranged from 0.10 to 0.16mm. Higher values of thickness were noticed by Chandla et al. (2017) for the films of amaranth starches from different cultivars. Water solubility of

edible film is the key factor in determining its applicability. High water solubility of film results in partial or complete dissolution of film rapidly while low water solubility slows the degradation of film. Heat moisture treated and oxidised starch films were intact after immersion in water for 24hr, while native amaranth starch film samples were partially dissolved. The water solubility of amaranth starch films ranged from 32.89 to 48.11% and significantly lower solubility of films was noticed in case of modified starches as compared with native starch of amaranth. Chandla et al. (2017) reported lower values of water solubility (33.64 to 37.56%) of films made from starches of amaranth from different cultivars as compared to solubility of native starch film of amaranth. Zavareze et al. (2012) observed decreased solubility of starch film made up of oxidised and hydrothermally modified starches of potato relative to solubility of native potato starch films. Fonseca et al. (2015) developed potato starch film and noticed lower water solubility of film prepared from starches oxidised with different levels of sodium hypochlorite as compared with films made from native starch of potato using similar concentration of starch. The reduced water solubility of the hydrothermally modified and oxidised amaranth starch films might be attributed to the increased interactions between amylose-amylose, amylose-amylopectin chains and strengthened intermolecular bonds promoted during modification treatments.

The tensile strength indicates the force applied at the break point of film. Considerably increased values of tensile strength were noticed for films of oxidized and heat-moisture treated amaranth starches as compared to films of native starch. The value of tensile strength of films made from native starch was 0.734MPa indicating

lower strength of films as compared with findings of Chandla et al. (2017) reported tensile strength ranged from 2.30 to 2.61MPa for films of amaranth starches of different cultivars. The discrepancy in mechanical strength of films prepared from amaranth starch in present study and previous reports might be due to the differences in concentration of starch used for film formation, thickness, film formation conditions like heating temperature and drying rate. Oxidised starch showed tensile strength value of 1.42MPa which was higher than that of native starch film samples. Hydrothermally modified starch showed the highest value (2.51MPa) of tensile strength of film among tested samples. It has been reported by Zavareze et al. (2012) that oxidation and heat moisture treatment of potato starch increased the tensile strength of the films from 3.53 to 5.25MPa and 3.53 to 6.07MPa, respectively. Increment in tensile strength of film following oxidation of potato starch with 1.0% active chlorine while decrement in the value of tensile strength of films was noticed for potato starches oxidised with 0.5 and 1.5% active chlorine (Fonseca et al., 2015). The mechanical properties of the starch films depend on various factors such as polymeric chain arrangement, molecular chain interactions, film thickness, quantity and type of the plasticizer, and relative humidity of the environment. Additional interaction among amylose and amylopectin molecules resulted from heat-moisture treatments could be the reason for increment of tensile strength of films. Zhang et al. (2009) suggested that carbonyl and carboxyl groups present in oxidised starch can form hydrogen bonds with amylose and amylopectin chains, and these bonds offer larger structural integrity in the polymer matrix, thus causing increased tensile strength of film.

Table.1: Moisture content, thickness, solubility, water vapour permeability and tensile strength of films of native and modified starches of amaranth

Treatments	Moisture content (%)	Thickness (mm)	Solubility (%)	WVPR (g.mm/m ² .day.kPa)	Tensile strength (MPa)
NS	13.21±0.10 ^a	0.158±0.00 ^a	48.11 ±0.34 ^a	6.88±0.03 ^a	0.734±0.00 ^a
Oxi-S	14.77±0.32 ^b	0.157±0.00 ^a	38.38±0.06 ^b	5.72±0.01 ^b	1.42±0.00 ^b
HMT (at 85°C)	15.60±0.17 ^c	0.161±0.00 ^b	32.89±0.56 ^c	7.20±0.03 ^c	2.51±0.00 ^c

All values are mean of triplicate determinations ± standard deviation mean. Values within same column with different letters are significantly different ($p \leq 0.05$). NS: native starch; HMTS: heat moisture treated starch; Oxi-S: oxidized starch; mm: millimetres; kPa: kilopascal; MPa: megapascal

Water vapour permeability evaluates of easiness with which moisture can permeate through the film. The water vapour permeability of film is required low to provide a barrier for the transfer of moisture between the food and

the surrounding atmosphere. Native and modified starches showed significantly different values of water vapour permeability of films. Heat-moistur treated starch showed higher water vapour permeability of film while oxidised starch showed lower values with respect to native starch of amaranth. Similar trend of increased water vapour permeability of hydrothermally modified starches and decreased values for oxidized starches was reported by Zavareze et al. (2012) for the potato starch films. Higher barrier property for moisture were recorded by Fonseca et

al. (2015) for oxidised potato starch films than that of native starch films, and increasing level of oxidation resulted in raising water barrier capacity of film during oxidation. On further oxidation, these carbonyl groups were transformed to carboxyl groups (hydrophilic) and hydrophilicity of oxidized starch increased significantly. Therefore, lower water vapour permeability of oxidized starch films in the present investigation could be attributed to the higher carbonyl groups (hydrophobic) in oxidized starch than native starch. In heat-moisture treatment, retrogradation in starch gel takes place due to interaction in amorphous region at initial stage followed by interaction in crystalline domains. As amaranth starch is waxy type, heat moisture treatment increased stiffness in the starch granules and caused lesser retrogradation (due to absence of amylose) resulted in loose packing of gelatinised granules offering space for mobility of water molecules, consequently more hydrophilic films formed with more water vapour permeability and stiffness. Thickness of film is an another important factor affecting

water vapour permeability and higher thickness of films in case of heat moisture treated starches might be the reason for more water vapour permeability than native starch film. Linearly increasing water vapour permeability with increasing thickness and hydrophilicity of starch films was recorded for different starches in literature (Cuq et al., 1996; Mali et al., 2004; Zavareze et al., 2012).

3.2 Color Parameters of Films

Color of the film is key factor influencing the appearance of product on which it is applied. Table 2 depicts the color parameters of films native and modified starch films of amaranth. Modification treatments of amaranth starch changed color of starch, consequently altered color (L^* , a^* and b^* parameter) of produced starch films. No significant difference was noticed in lightness of native and modified starch films indicated by statistically similar L^* values. Slight greenish shade was noticed in all film indicated by negative values of a^* ranging from -0.25 to -0.40 with the highest value exhibited by oxidised starch film.

Table.2: Color properties of films of native and modified starches of amaranth

Treatments	L^*	a^*	b^*
NS	83.48±0.02 ^a	-0.25±0.01 ^a	3.26±0.04 ^a
Oxi-S	83.55±0.23 ^a	-0.40±0.02 ^c	2.80±0.03 ^c
HMT (at 85°C)	83.61±0.28 ^a	-0.26±0.01 ^a	3.32±0.02 ^a

All values are mean of triplicate determinations ± standard deviation mean. Values within same column with different letters are significantly different ($p \leq 0.05$). NS: native starch; HMTS: heat moisture treated starch; Oxi-S: oxidized starch; L^* : black to white; a^* : green to red; b^* : blue to yellow

Yellowness in starch films represented by positive b^* values ranged from 2.8 to 3.32 with the highest value observed for hydrothermally modified starch films, however, it was not statistically dissimilar to the value of native starch film. Change in color of heat moisture treated starch films could be attributed to the occurrence of Millard reaction during modification treatment of starch. Improved lightness of oxidised starch films might be attributed to the increased whiteness of starch by bleaching action of chlorine during oxidation. Fonseca et al. (2015) reported comparatively higher L^* values ranging from 87.6 to 89.7 for starch films prepared from native and oxidised starches of potato.

IV. CONCLUSION

Amaranth starch was found interesting material for film formation. Native and modified starches of amaranth produced biodegradable films with different characteristics. Both the modification treatments resulted in increased tensile strength of films. Water vapour

permeability of films increased in heat moisture treated while decreased in oxidised starch film samples. Overall, amaranth starch films were transparent, continuous and had good tensile strength.

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